U.S. FISH AND WILDLIFE SERVICE
SPECIES ASSESSMENT AND LISTING PRIORITY ASSIGNMENT FORM

SCIENTIFIC NAME: *Crystallaria cincotta*

COMMON NAME: Diamond Darter

LEAD REGION: 5

INFORMATION CURRENT AS OF: May 2010

STATUS/ACTION: Candidate; Non-petitioned

ANIMAL/PLANT GROUP AND FAMILY: Fish, Percidae

HISTORICAL STATES/TERRITORIES/COUNTRIES OF OCCURRENCE: Kentucky, Ohio, Tennessee, and West Virginia.

CURRENT STATES/COUNTIES/TERRITORIES/COUNTRIES OF OCCURRENCE: West Virginia

LAND OWNERSHIP: The only extant population of the diamond darter occurs within the portion of the Elk River below Sutton Dam in Kanawha and Clay counties, West Virginia. Of the approximately 708,500 acres (286,730 hectares) within this watershed (Strager 2008, pg 13), 97% is privately owned. Public lands include two wildlife management areas, Wallback and Morris Creek, totaling 21,632 acres (8754 hectares), that are either owned or leased by the West Virginia Division of Natural Resources (WVDNR 2007, pages 22 & 34), and Coonskin Park, a recreational facility located along the Elk River, near Charleston, WV. This park, which covers approximately 1000 acres (405 hectares), is owned by Kanawha County and includes a golf course, pool, recreational ball courts, and woodland areas (Kanawha County Parks and Recreation 2008, page 1).

LEAD REGION CONTACT: Martin Miller, (413) 253-8615, Martin_Miller@fws.gov

LEAD FIELD OFFICE CONTACT: West Virginia Field Office, Barbara Douglas, (304) 636-6586 x19, Barbara_Douglas@fws.gov

BIOLOGICAL INFORMATION

Species Description

The diamond darter is a member of the Perch family (Percidae), a group characterized by the presence of a dorsal fin separated into two parts, one spiny and the other soft (Kuehne and Barbour 1983, page 1). The darters differ from other percids in being much smaller in overall size and having a more slender shape. Some darters, including those in the genus *Crystallaria,* lack a swim bladder. This characteristic increases the density of the fish and facilitates their ability to remain near the bottom with little effort (Evans and Page 2003, page 64).

The diamond darter is overall translucent with silvery white ventral side of the body and head and four wide olive-brown saddles on the back and upper side (Welsh et al. 2008, page 1). Between the saddles, olive brown melanophores on scale margins produce a fragmented cross-hatch pattern. A preorbital blotch is dark and distinctly separated from the anterior margin of the orbital rim. The mid-lateral pigmentation includes 12-14 oblong olive-brown blotches overlain by an iridescent olive green stripe. Fins are clear with the exception of sparse pigmentation on the caudal fin. Documented standard lengths range from 73 to 77.3 millimeters (mm) (2.9 – 3.0 inches) (Welsh and Wood 2008, pages 64-66)

Characteristics that distinguish the diamond darter from the related crystal darter (*C. asprella*) that occurs in the Gulf Coast, and the Mississippi and Wabash rivers include the presence of a larger gape width approximately equal to or exceeding the inter-pelvic fin base width; a preorbital blotch distinctly separate from the anterior orbital rim; pelvic fins distinctly falcate in both sexes; cheek scale rows reduced (most frequently 2); opercle scale rows reduced (most frequently 2); a high count of mid-lateral blotches (most frequently 13); a low count of anal-fin rays (most frequently 13); a low count of dorsal-fin spines (most frequently 12), and a high count of scales below the lateral line (most frequently 11) (Welsh and Wood 2008, page 66).

<u>Taxonomy</u>

Previously, *Crystallaria* was regarded as a subgenus within *Ammocryta* (Cincotta and Hoeft, 1987 page 133; Simons 1991, page 934). However, in a phylogenetic analysis based on morphology, Simons (1991) elevated *Crystallaria* to generic status based on its monotypic origin. This treatment has been adopted in other subsequent works (Page and Burr 1991, Simons 1992, and Wiley 1992 in NatureServe, 2008, page 1). Allozyme data also seem consistent with this arrangement (Wood and Mayden 1997 in NatureServe 2008, page 1).

When it was first collected from the Elk River, West Virginia in 1980, the specimen was identified and reported as the crystal darter (*Crystallaria* ne: *Ammocrypta asprella*) (Cincotta and Hoeft 1987, pages 133-136). This was the first collection of this species from the Ohio River Basin in 41 years and the first time it was ever collected in West Virginia (Cincotta and Hoeft, 1987 page 133). Although the diagnostic characteristics of the specimen were within those described for the crystal darter by Page (1983), even at the time of collection some researchers believed that the species, as then recognized, actually constituted more than one subspecies or species (Cincotta and Hoeft 1987, page 134), particularly given the disjunct nature of existing crystal darter populations.

In order to explore this possibility, Wood and Raley (2000) evaluated the genetic variation of five crystal darter populations by sequencing the mitochondrially-encoded cytochrome b gene. Individuals from populations in the Pearl River in Louisiana, the Cahaba River in Alabama, the Saline River in Arkansas, the Zumbro River in Minnesota, and the Elk River in West Virginia were evaluated. Phylogenetic analysis was conducted on these crystal darter specimens as well as individuals from eight other darter species (Wood and Raley 2000, page 20). This study found that there was an 11.2-11.8 percent difference between the cytochrome b sequence of the Elk River crystal darter population and all other crystal darter populations evaluated (Wood and Raley 2000, page 24). This was one of the highest divergence values in cytochrome b ever reported for a fish species (Wood and Raley 2000, page 24), and was more typical of interspecific or intergeneric differences (Song et al. 1998 in Wood and Raley 2000, page 24).

Because differentiation observed at a single gene region is generally not considered sufficient evidence to establish taxonomic status, additional analyses were initiated by Morrison et al. (2006, page 129). In that study, nucleotide variation at the mitochondrial control region (mtDNA CR) and a nuclear S7 ribosomal gene intron were compared to provide independent verification of phylogeographic results between individuals collected from the same five disjunct crystal darter populations previously surveyed (Morrison et al. 2006, page 129). In addition, morphometric measurements and meristic[1] counts between individuals from these populations were compared (Morrison et al. 2006, page 130). The results of this study confirmed the conclusions of Wood and Raley (2000) in regard to the Elk River populations. The magnitude of divergence between the Elk River population and the other populations sampled, as estimated from mtDNA CR data, was similar in magnitude to mtDNA divergences measured between recognized species of darters and was an order of magnitude greater than some mtDNA CR divergence estimates for recognized subspecies (Morrison et al. 2006, page 139). Morphometric data were also consistent with molecular data regarding the distinctiveness of the Elk River population (Morrison et al. 2006, page 129). The study concluded that the Elk River group likely constituted a distinct species (Morrison et al. 2006, page 143).

Welsh and Wood (2008) conducted additional morphological comparisons between *Crystallaria* populations from 18 rivers within the Ohio River drainage, the upper, middle, and lower Mississippi River drainages, and the Gulf Coast (Welsh and Wood 2008, page 63). This evaluation included specimens from extant populations, as well as museum specimens from currently extirpated populations that were gathered during the late 1800s to early 1900s. Nine specific morphological characteristics were identified that distinguish the Elk River population from other populations of the crystal darter (see Species Description section). Based on the results of this analysis, and the previous genetic studies, Welsh and Wood (2008, pages 62-68) formally named and described the Elk River population of the crystal darter as a separate and distinct species, the diamond darter (*Crystallaria cincotta*) (Welsh and Wood 2008, pages 62-68). The paper further identified that specimens from extirpated populations within the Cumberland, Green, and Muskingum Rivers within the Ohio River Basin were consistent with the characteristics defined for the diamond darter, thus establishing the extent of the species' historical range. The current range of the crystal darter does not appear to overlap with the diamond darter and includes Alabama, Florida, Illinois, Louisiana, Mississippi, Minnesota, and Missouri (Welsh and Wood 2008, pages 62-68; Grandmaison et al. 2003, page 6).

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1 Meristics are systematic counts of fish characteristics such as the number of scales along the lateral line or the number of rays in the anal fin.

We carefully reviewed the available taxonomic information summarized above and conclude that the species is a valid taxon based upon considerations of genetic and morphological characteristics.

Habitat/Life History

Due to its rarity, little research exists on the natural history of this species (Osier 2005, page 10). However, in some cases, potential characteristics can be inferred from the information available on the closely-related crystal darter, as noted below.

The diamond darter is a species that inhabits moderate to large, warm water streams with moderate current and clean sand and gravel substrates (Simon and Wallus 2006, page 52). In the Elk River, the diamond darter has been collected from riffles and pools at depths between 0.5 to 1.5 meters (1.6 to 4.9 feet (ft)) and in moderate to strong velocities. Substrates in diamond darter capture areas are typically sand and gravel (Osier 2005, page 11) and are less embedded than other surrounding areas (Welsh et al. 2004, page 7). Mean water velocities around one capture site ranged from 10 to 45 centimeters/second (s) (.33 to 1.48 ft/s) (Welsh et al. 2004, page 6). The diamond darter may use habitats that are similar to those described for the crystal darter (Welsh et al. 2008, page 1). Swift currents result in the clean swept substrates and lack of silt commonly reported in documented crystal darter habitat (Osier 2005, page 11). Many studies have found that the crystal darter does not occur in areas with large amounts of mud, clay, detritus, or submerged vegetation (NatureServe 2008, page 1; George et al. 1996, page 71; Shepard et al. 1999 in Osier 2005, page 11). In Arkansas, crystal darter capture areas had dissolved oxygen that ranged from 6.81 to 11.0 parts per million; pH levels of 5.73 to 6.6; specific conductivity of 175-250 umhos; and water temps of 14.5 – 26.8$^{o\circ}$C (58 – 80$^{o\circ}$F) (George et al. 1996, page 71).

Diamond darters recently observed in captivity were crepuscular (more active at dusk and dawn). Two of the three individuals observed were typically seen either completely buried in the sand during the day or partially buried with only the head (eyes and dorsal snout) out of the sand. The third (largest) individual was often on top of the sand during the day, and only occasionally buried in the sand. However, all three were often on top of the sand at night time (Welsh 2009c, page 1). Burying occurred by the individual rising slightly up above the substrate and then plunging headfirst into the sand and using its caudal motion to burrow (Welsh 2009c, page 1). This type of burying behavior has also been reported in the crystal darter (NatureServe 2008, page 1; Osier 2005, page 11). These observations are also consistent with the results of both diamond darter and crystal darter sampling efforts, in that most researchers found that these fish were more often collected at dusk or during the night (Welsh 2008, page10).

It is expected that, similar to the crystal darter, the diamond darter is a benthic invertivore (an animal that feeds primarily on stream bottom dwelling invertebrates) (NatureServe 2008, page 8). Three diamond darters kept in captivity were fed frozen brine shrimp and blood worms, and seemed to prefer the bloodworms (Welsh 2009c, page1). These fish did not swim up into the water column to get food but often rested on the bottom of the tank and took food from slightly above their position, in front of them, or off the bottom (Welsh 2009c, page 1). Although not observed during the few months that the diamond darters were observed in captivity (Welsh 2009c, page 1), *Crystallaria* may use an ambush foraging tactic by burying in the sand and darting out at prey (NatureServe 2008, page1; Robinson 1992 and Hatch 1997 in Osier 2005, page 12-13). Crystal darters eat midge and caddis fly larvae, and water mites in lesser quantities (Osier 2005, page 13). Juvenile and young crystal darters feed on immature stages of aquatic insects such as mayflies, crane flies, black flies, caddis flies, and midges (Simons and Wallus 2006, pages 56-57).

Very little information is available on reproductive biology and early life history of the diamond darter (Ruble & Welsh 2010, page 1; Welsh et al. 2008, page 1), and to-date only one young-of-the-year of this species has been found in the wild. We have not been able to obtain specific information on this collection, which probably occurred in 2007 in the Elk River near the confluence with the Kanawha River, West Virginia (Cincotta 2009a, page 1). However, research on reproductive biology of the species was recently initiated by Conservation Fisheries Inc. (CFI) in partnership with the U.S. Geological Survey (USGS) West Virginia Cooperative Fish and Wildlife Research Unit at West Virginia University (WVU). Five individuals, consisting of at least three females and one male, are currently being held in captivity at the CFI facility and are maintained to simulate stream conditions. Temperature and daylight are also adjusted throughout the seasons in an environment that simulates natural fluctuations that would be experienced in the wild (Ruble & Welsh 2010, page 2). During the winter, when temperatures are below 15°C (59°F), activity decreased and the darters were commonly observed buried in the sand. Females began to show signs of being gravid between late March and May. Checks for eggs and larvae began when temperatures were consistently above 18°C (64.4°F). The first spawning occurred in April 2010. Eggs were observed on April 8, 2010, and on April 9, 2010, larvae hatched with heavy yolk sacs. Once temperatures were above 21°C (70°F), diamond darters consistently were observed above the sand substrate during both the day and night (Ruble & Welsh 2010, page 2).

There is also some information available on reproduction of the sister species, the crystal darter (Welsh et al. 2008, page1). In Arkansas, the reproductive season was from late January through mid-April, roughly correlating with early April in the Ohio River Basin (George et al. 1996, page 75; Simon and Wallus 2006, page 52). Evidence suggests that females are capable of multiple spawning events and producing multiple clutches of eggs in one season (George et al. 1996, page 75). Spawning occurs in the spring when the darters lay their eggs in side channel riffle habitats over sand and gravel substrates in moderate current. Water temperatures at spawning were 12-13°C (53.6 – 55.4°F) (Simon and Wallus 2006, page 52). Adult darters do not guard their eggs (Simon and Wallus 2006, page 56). Embryos develop in the clean interstitial spaces of the coarse substrate (Simon and Wallus 2006, page 56). After hatching, the larvae are pelagic drifters and occur within the water column during dusk and at night (Simon and Wallus 2006, page 56; Osier 2005, page 12; NatureServe 2008, page 1).

Life expectancy for the crystal darter has been commonly reported to range from 2-4 years (Osier 2005, pages 10-11), although some authors have suggested the potential to live up to seven years (Simon and Wallus 2006, page 52). In Arkansas, sexual maturity for the crystal darter may occur during the first year, with the first spawning occurring the season after hatching. However in the Ohio River Basin this may not occur until age three (George et al. 1996, page 75; Simon and

Wallus 2006, page 52). Reported differences in age and size at maturity between northern and southern populations of crystal darters have been attributed to environmental differences, such as flow regimes, photoperiod, and temperature, with southern populations maturing and reproducing at an earlier age and thus having shorter lifespans (George et al. 1996, pages 75-76).

Historical Range/Distribution

As shown in the table below, historic records of the species indicate that it was distributed throughout the Ohio River Basin and that the range included the Muskingum River, Ohio; the Ohio River, Ohio; the Green River, Kentucky; and the Cumberland River Drainage, Kentucky and Tennessee. There is some difference of opinion as to how common the species was during the early portions of the 1900s. Trautman (1981, page 645) suggests that it is quite probable that before 1900 the species was well distributed in the lower reaches of the southern Ohio tributaries and the Ohio River. However in 1892, Woolman (in Cicerello 2003, page 6) noted that the species was likely neither widely distributed, nor common anywhere in Kentucky.

Historic Diamond Darter Collections					
Date	**State**	**River**	**General Location**	**Citation**	**Notes**
1888	OH	Muskingum	near Beverly, Washington Co.	Trautman 1981, page 645; Kibbey 2008, page 1	*
1899	OH/KY	Ohio	near Ironton, Lawrence Co. OH/Greenup Co., KY	Trautman 1981, page 645; Kibbey 2008, page 1; Clay 1975 page 315; KSNPC 1991, page 1	*
pre-1899	KY/IN	Ohio	near Rising Sun, IN; Boone Co., KY	Jordan 1899 in KSNPC 1991, page 3	
1890	KY	Green River	near Greensburg, Green Co.	Clay 1975, page 314; KSNPC 1991, pages 4 & 5	2 collected*
1929	KY	Green River	Mammoth Cave, Edmonson Co.	Clay 1975, page 315; KSNPC 1991, page 6	2 collected*
1890s	KY	Cumberland	near Kuttawa, Lyon Co.	KSNPC 1991, page 2; Burr and Warren 1986, page 285	*
1939	TN	Cumberland	Clay Co.	Shoup et al. 1941 in Etnier and Starnes 1993, page 443	
1939	TN	Roaring River (tributary to Cumberland River)	Jackson Co.	Shoup et al. 1941 in Etnier and Starnes 1993, page 443	

1870	TN/KY	Big South Fork of the Cumberland	Scotts Co.,TN near KY state border	Comiskey and Entier 1972 in Entier and Starnes 1993, page 443	

***s indicate that these specimens are still extant in museums and were confirmed as C. cincotta in the analysis that described and differentiated between that species and C. asprella (Welch and Wood, 2008a). The other specimens are no longer extant and/or could not be located. It is assumed that these occurrences are also C. cincotta because they occur within the watershed upstream of and/or in close proximity to other confirmed specimens of the species.**

Current Range/Distribution

The species is currently only known to exist within a 36 km (22.4 mile) section of the lower Elk River in Kanawha and Clay counties, West Virginia, and is considered extirpated from the remainder of the Ohio River Basin (Wood and Welsh 2008, page 1 and 5; Warren et al. 2000 in Cicerello 2003, page 3). The species was first collected from the Elk River in November 1980 when one individual was collected during boat electroshocking surveys conducted near Mink Shoals in Kanawha County (Cincotta and Hoeft 1987, page 133). This collection marked the rediscovery of the species in the Ohio River Basin, where it formerly had been considered extirpated from all states in which it had previously been recorded (Cincotta and Hoeft 1987, page 133-134). The species has not been collected since 1899 in Ohio, 1929 in Kentucky, and 1939 in Tennessee (Hatch 1998 in Grandmaison et al. 2003, page 6). Figure 1 shows the location of current and historic collections of the species.

Trautman (1981, page 645) suggests that increased silt load and subsequent smothering of suitable habitats likely caused the extirpation of the species from the State of Ohio by 1925 and that "the habitat of few other Ohio fishes seemed so vulnerable to annihilation" (Trautman 1981, page 646). In addition, researchers at the Ohio State University have conducted extensive sampling in the Ohio River and its tributaries starting with Ed Wickliff in the 1920s and continuing through the present (Ohio State University 2008, page 1; Kibbey 2008, page 1). Despite semi-annual survey efforts in likely diamond darter habitat, such as the riffles below Devola Dam on the Muskingum River, no additional diamond darters have been located (Kibbey 2008, page 1). The Midwest Biodiversity Institute has also conducted recent surveys in the Muskingum River using both trawls and electroshocking. These surveys also failed to locate any *Crystallaria* species (Kibbey 2008, page1). Furthermore, despite conducting over 20,000 individual sampling events at over 10,000 locations throughout the State of Ohio, including in both large rivers and small creeks, the Ohio Environmental Protection Agency has never collected any *Crystallaria* species (Mishne 2008, page1). As a result of these efforts, the species is considered extirpated from both the State of Ohio and the Ohio River (Mishne 2008, page 1; Trautman 1981, page 646). Pearson and Krumholtz (1984, page 252) state that the chances of the diamond darter currently being present in the entire mainstem Ohio River are "remote at best."

The species is also considered extirpated from the State of Kentucky (Burr and Warren 1986, page 285; Evans 2008, page 1). The State has been fairly well surveyed by numerous researchers without resulting in any recent collections of the species (Evans 2008, page 1). All historical Green River sites have been repeatedly but unsuccessfully sampled for the diamond darter (Cicerello 2003, page 6). Both the Kentucky State Nature Preserves Commission (KSNPC) and Southern Illinois University have conducted surveys targeting the species throughout the upper portion of the Green River Basin (Cicerello 2003, page 6). Most recently in 2007, the Kentucky Department of Fish and Wildlife Resources, the Missouri Department of Conservation, and KSNPC sampled below Lock and Dam 5 and 6 on the Green River, as well as in river reaches downstream of the dams using a Hertzog trawl (Evans 2008, page 1). The Kentucky Department of Fish and Wildlife Resources has also done some site monitoring in the Green River at three sites below Green River dam and has not collected the species.

The diamond darter has not been documented to occur in Tennessee since 1939, and all previous records of the species within the State were from within the Cumberland River drainage (Etnier and Starnes 1993, page 443). Starting in the 1950s, dams were installed on the mainstem Cumberland River that impounded much of its entire length from Barkley Dam in Kentucky to Cumberland Falls near the headwaters (Tennesee Wildlife Resources Agency (TWRA) 2005, page 14). This dramatically altered most of the riverine habitat qualities that made the river suitable for the diamond darter and likely resulted in the extirpation of the species (TWRA 2005, page 14; Etnier and Starnes, 1993, page 443; Saylor, 2009, page 1). Cold water discharges from many of these dams have changed the natural temperature regimes so that the river no longer functions as a warm water fishery (TWRA 2005, page 14; Fiss 2009, page 1). In addition, when the Cumberland River impoundments were being constructed, a fish barrier was installed near the mouth of the Roaring River in order to keep species that might frequent the impoundments, such as carp, from moving into the Roaring River, thus impeding any connectivity between the two systems (Fiss 2009, page 1). Currently the Cumberland River watershed is subject to incompatible pasture and grazing management practices, forest clearing, heavy navigation and recreational use, active mining, historic mining and acid mine drainage issues, oil and gas drilling, lack of riparian buffers, and poor stormwater and wastewater management (TWRA 2005, page 135-136). Despite these threats, the Cumberland aquatic region still contains some of the highest diversity of fish, mussel, and crayfish species in North America (TWRA 2005, page 14), and some ichtyologists have suggested that there is a “remote possibility” that the diamond darter may still exist in the cleaner large tributaries of the Cumberland or the lower Tennessee Rivers (Etnier and Starnes 1993, page 444). Therefore some targeted sampling may be warranted (Fiss 2009, page 1). Although the gear used during some of these surveys was not targeted towards capturing the diamond darter (Fiss 2009, page 1), the TWRA has conducted 111 fish survey samples from 1996 to 2007 throughout the Cumberland River system, and has no records of recent diamond darter captures (Kirk 2009, page 1). Despite extensive sampling in the Duck River as well as the Blood and Big Sandy Rivers, there are no current or historic records of the diamond darter in those rivers either (Saylor 2009, page 1).

<u>Population Estimates/Status</u>

Sufficient information is currently not available to develop an overall population estimate for the species. However, survey records confirm that the species is extremely rare. Fish surveys have been conducted in the Elk River in 1936, 1971, 1973, 1978 – 1983, 1986, 1991, 1993, 1995, 1996, and every year since 1999 (Welsh et al. 2004, pages 17-18; Welsh 2008, page 2; Welsh 2009a, page 1). Survey methods included backpack and boat electrofishing, underwater observation, kick seine, and bag seines (Welsh et al. 2004, page 4). Starting in early 1990s, the timing of sampling and specific methods used were targeted towards those shown to be efficient at capturing similar darter species during previous efforts (Welsh et al. 2004, page 4-5; Hatch 1997, Shepard et al. 1999, and Katula 2000 in Welsh et al. 2004, page 9). Despite these extensive and targeted survey efforts within the species' known range and preferred habitat in the Elk River, only 19 individuals have been collected in the last 30 years since the species was first collected in the Elk River. Twelve individuals were collected between 1980 and 2005, and an additional 7 specimens have been collected since that time (SEFC 2008 page 10, Cincotta 2009a, page 1; Cincotta 2009b, page 1; Welsh 2009b, page 1, Ruble & Welsh 2010, page 2). Welsh et al. (2004, page 8) concludes that the number of individuals in the Elk River is likely small given the low catch per unit effort in both previous and recent data. Independent publications that have evaluated the status of the species further corroborate the rarity of the species. For example, the diamond darter was recently highlighted as a Threatened Fish of the World (Welsh et al. 2008, pages 1-2) and was listed by the Southeastern Fishes Council as one of the 12 most imperiled fishes (i.e., "desperate dozen") of the southeastern United States (SEFC 2008, pages 2-3).

THREATS

A. <u>The present or threatened destruction, modification, or curtailment of its habitat or range</u>.

As indicated by the continued persistence of the diamond darter, the Elk River in West Virginia currently provides overall high quality aquatic habitat. The Elk River is one of the most ecologically diverse rivers in the State (Green 1999, page 2) supporting over 100 species of fish and 30 species of mussels, including three federally-listed mussel species (Welsh 2009a, page 1). The river, including those portions that are within the range of the diamond darter, is listed as a "high quality stream" by the West Virginia Division of Natural Resources (WVDNR 2001, pages 1, 2 & 5). Streams in this category are defined as having "significant or irreplaceable fish, wildlife, and recreational resources" (WVDNR 2001, page iii). In an evaluation of the watershed, the West Virginia Department of Environmental Protection noted that all four sampling sites within the mainstem of the Elk River scored well for benthic macroinvertebrates on the West Virginia Stream Condition Index, with results of 77 or higher out of a potential 100 points (WVDEP 1997, page 41). Criteria for placement on the high quality streams list are based solely on the quality of fisheries populations and the utilization of those populations by the public and do not include water quality or threats to the watershed (WVDNR 2001, page 36; Brown 2009, page 1). Consequently, despite the high quality of the fishery populations there are continuing and pervasive threats within the watershed. In fact, the WVDEP evaluation also noted that because larger rivers offer a wider variety of microhabitats, the high benthic macroinvertebrate scores may mask some degradation in water quality (WVDEP 1997, page 41). Noted threats to the watershed include coal mining, oil and gas development, sedimentation/erosion, timber harvesting, water quality degradation, and poor wastewater treatment (Strager 2008, pages 1-39; WVDEP 1997, page 15; WVDEP 2008b, pages 1-2). Many sources have recognized that *Crystallaria* species appear to be particularly susceptible to habitat alterations and changes in water quality. Threats similar to those experienced in the Elk River watershed have likely contributed to the extirpation of *Crystallaria* within other watersheds (Trautman 1981, pages 24-29, 646; Clay 1975, page 315; Grandmaison 2003, pages 16-19). In addition, the current range of the diamond darter is restricted and isolated from other potential and historical habitats by impoundments.

Coal Mining

As shown in Figure 2, coal mining occurs throughout the Elk River watershed, with most of the active mining occurring towards the southern extent of the watershed (Strager 2008, page 17). As of January 2008 there were over 13,000 acres (5,260 hectares) of actively-mined areas including 91 surface mine permits, 79 underground mine permits, 3,339 acres (1,351 hectares) of valley fills, 362 miles (582 km) of haul roads, 239 miles (385 km) of mine drainage structures, 473 NPDES sites associated with mines, and 3 mining related dams (Strager 2008, pages 19-21). There are also 1,519 acres (615 hectares) of abandoned mine lands and 155 mine permit sites that have forfeited their bonds and not adequately remediated the sites (Strager 2008, page 18). Approximately 47% of the entire Elk River watershed is within the area that the U.S. Environmental Protection Agency has identified potentially being subject to mountain top removal mining activities (Strager 2008, page 17).

Coal mining can contribute significant amounts of sediment and degrade water quality in streams. For example, in the Jacks Run Watershed, a tributary to the Elk River, one third of the entire watershed had been recently cleared for mining. In a sampling site downstream of mining, the WVDEP noted embedded substrate with dark silt, most likely from manganese precipitate or coal fines, and that benthic scores indicated severe impairment (WVDEP 1997, page 60). Another Elk River tributary (Blue Creek) had low pH levels associated with contour mining and acid drainage and three sample sites had pHs of 4.2 or less (WVDEP 2008b, page 6; WVDEP 1997, page 47). At pH levels of 5 or less most fish eggs cannot hatch (USEPA 2009, page 2). Sampling sites in the Buffalo Creek drainage below a large mining reclamation site were in violation of acute aluminum and manganese water quality criteria, habitat quality was poor, and the substrate was heavily embedded with coal fines and clay. Other sites in the watershed where topographic maps showed extensive surface mining, had pH readings of 4.7, elevated aluminum levels, and benthic communities that were dominated by acid-tolerant species (WVDEP 1997, pages 4, 56-57).

A USGS study of the Kanawha River Basin, which includes the Elk River, found that streams draining basins that have been mined since 1980 showed increased dissolved sulfate, decreased median bed-sediment particle size, and impaired benthic-invertebrate communities when compared to streams not mined since 1980. Stream-bottom sedimentation in mined basins was also greater than in undisturbed basins (USGS 2000, page 1). In streams that drained areas where large quantities of coal had been mined, the benthic invertebrate community was impaired in comparison to rural parts of the study area where little or no coal had been mined since 1980 (USGS 2000, page 7). That report notes that benthic invertebrates are good indicators of overall stream water quality and that an impaired invertebrate community indicated “more than a disruption in the aquatic food web— it indicates that stream chemistry and (or) physical habitat are impaired” (USGS 2000, page 8). In another study that specifically evaluated fish data, the

Index of Biotic Integrity (IBI) scores at sites downstream of valley fills were significantly reduced by an average of 10 points when compared to unmined sites, indicating that fish communities were degraded below mined areas (Fulk et al. 2003, page iv). In addition, that study noted a significant correlation between the number of fishes that were benthic invertivores and the amount of mining in the study watershed, and that the number of those types of fish species decreased with increased mining (Fulk et al. 2003, pages 41-44). As described above in the Life History section, the diamond darter is a benthic invertivore. The effects described above are often more pronounced in smaller watersheds (USGS 2000, page 1) that do not have the capacity to buffer or dilute degraded water quality. Because the mainstem Elk River drains a relatively large watershed, these types of adverse effects are more likely to be noticed near the confluences of tributaries that are most severely altered by mining activities.

In addition to the chronic sediment release and water quality effects from coal mine areas, there exists the potential for failure of large-scale mine waste (coal slurry) impoundment structures contained by dams, which could release massive quantities of mine wastes that could cover the stream bottoms. There are currently two coal slurry impoundments within the Elk River watershed. These impoundments have a capacity of 221,000,000 and 50,000,000 cubic feet (6,258,023 and 1,415,842 m^3). The larger structure covers 48 acres (19 hectares) and is considered a "class C" dam which could result in the loss of human life and serious damage to homes, and industrial and commercial facilities in the event of failure (Strager 2008, pages 21-22). A third coal refuse disposal impoundment is permitted and planned for construction with an additional 1,936,000 cubic feet (991,090 m^3) of capacity (Fala 2009, page 1; WVDEP 2009, page 1). These three impoundments are on tributaries of the Elk above the reach of river known to support the diamond darter. In October 2000, a coal slurry impoundment near Inez, Kentucky breached, releasing almost 35,000,000 cubic feet of slurry into the Big Sandy Creek Watershed. "The slurry left fish, turtles, snakes and other aquatic species smothered as the slurry covered the bottoms of the streams and rivers and extended out into the adjacent flood plane" (USEPA 2001a, page 2). Over 100 miles of stream were impacted by the spill (USEPA 2001a, page 2). If a similar dam failure were to occur in the Elk River watershed, it could have detrimental consequences for the diamond darter population.

There is also a potential for abandoned underground mines to fill with water and "blow out" causing large discharges of sediment and contaminated water. Similar events have happened recently in nearby areas, including one in Kanawha County, West Virginia in April 2009 that discharged "hundreds of thousands of gallons of water" onto a nearby highway, and caused a "massive earth and rock slide" (Marks 2009, page 1). A second situation occurred in March 2009 in Kentucky where water from the mine portal was discharged into a nearby creek at an estimated rate of 10,000 gallons a minute (Associated Press 2009, page 1). In addition to the increased levels of sediment and potential smothering of stream habitats, discharges from abandoned mine sites often have elevated levels of metals and low pH (Stoertz et.al. 2001, page 1).

Oil and Gas Development

The Elk River watershed is also one of the more densely drilled areas of the State with over 6,000 oil or gas wells in the watershed as of December 2007. As shown in Figure 3, the lower section of the Elk River, which currently contains the diamond darter, has the highest concentration of both active and total wells in the watershed with over 2,189 active wells and 590 abandoned wells (Strager 2008, pages 15-16). Although limited data are available to quantify potential impacts, development of oil and gas resources can increase sedimentation rates in the stream and degrade habitat and water quality in a manner similar to that described for coal mining. Oil and gas wells can specifically cause elevated chloride levels, and the erosion of roads associated with these wells can contribute large amount of sediment to the streams (WVDEP 1997, page 54).

WVDEP sampling sites within Summers Fork, a tributary to the Elk River with a "high density of oil and gas wells," had elevated chloride and conductivity levels as well as impaired benthic invertebrate scores despite "good benthic substrate" (WVDEP 1997, page 52). Factors cited as potentially contributing to the impaired benthic invertebrate scores at sample sites within the Buffalo Creek watershed included oil compressor stations next to the creek, pipes running along the bank parallel to the stream, and associated evidence of past stream channelization (WVDEP 1997, page 55). The WVDEP estimates the size of the average access road associated with an oil or gas well to be 1,300 feet (396 m) long by 25 feet (7.6 m) wide or approximately ¾ acre (.30 hectare) per well site (WVDEP 2008b, page 10). If each of the wells in the watershed has this level of disturbance, there would be over 4,500 acres (1,821 hectares) of access roads contributing to increased sedimentation and erosion in the basin. High levels of siltation have been noted in the impaired sections of the Elk River (USEPA 2001b, pages 3-6). Oil and gas access roads have been identified as a source that contributes "high" levels of sediment to the Elk River (USEPA 2001b, pages 3-7). Lack of road maintenance, improper construction, and subsequent use by the timber industry and all-terrain vehicle uses can increase the amount of erosion associated with these roads (WVDEP 2008b, pages 5-6).

Siltation (Sedimentation)

Excess siltation has been specifically noted as a threat to the Elk River system. Portions of the lower Elk River were previously listed as impaired due to elevated levels of iron and aluminum (Strager 2008, page 36; USEPA 2001b, page 1-1; WVDEP 2008b, page 1; WVDEP 2008a, page 18). The WVDEP has since revised those water quality criteria in order to address bioavailability of those metals, and the Elk River is therefore no longer listed as impaired for these constituents (WVDEP 2008a, page A-2). However, at the time that the previous criteria were in place, the WVDEP noted that impairment due to metals usually indicates excess sediment conditions (WVDEP 2008b, page 5), and identified coal mining, oil and gas development, timber harvesting, all-terrain vehicle usage, and stream bank erosion as sources of increased sedimentation within the Elk River watershed (USEPA 2001b, pages 1-1, 3-4 & 6; WVDEP 2008b, page 1). Within two subwatersheds that make up approximately 11% of the total Elk River Watershed area, the WVDEP identified 269 miles (433 km) of unimproved dirt roads and 47 miles (76 km) of severely eroding streambanks (WVDEP 2008b, page 5). There was also an estimated 3,283 acres (1328 hectares) of lands being actively timbered in those two watersheds in 2004 (WVDEP 2008b, page 6). Although data on timber harvesting for the entire Elk River watershed are not available, it is likely that these types of activities are common because there are 11 known sawmills within the watershed and the forested land is the predominant land-use category in the area (Strager 2008, pages 13 & 29).

Siltation has long been recognized as a pollutant that alters aquatic habitats by reducing light penetration, changing heat radiation, increasing turbidity, and covering the stream bottom (Ellis 1936 in Grandmaison et al. 2003, page 17). Increased siltation has also been shown to abrade and or suffocate bottom dwelling organisms, reduce aquatic insect diversity and abundance, and, ultimately, negatively impact fish growth, survival, and reproduction (Berkman and Rabeni 1987, page 285). The complexity and abundance of interstitial spaces is reduced dramatically with increasing sediment inputs as substrate embededness increases, and consequently, the amount and complexity of interstitial refugia and the amount of suitable breeding microhabitat for species such as the diamond darter is reduced (Bhowmik and Adams 1989, Waters 1995, Kessler and Thorp 1993, Osier and Welsh 2007 all in USFWS 2008, pages 15-16). Siltation directly affects the availability of food for the diamond darter by reducing the diversity and abundance of aquatic invertebrates on which the diamond darter feeds (Powell 2003, pages 34-35), and by increasing turbidity, which reduces foraging efficiency (Berkman and Rabeni 1987, pages 285-294). Research has found that when the percentage of fine substrates increases in a stream, the abundance of benthic insectivorous fishes decreases (Berkamn and Rabeni 1987, page 285).

Many researchers have noted that *Crystallaria* species are particularly susceptible to the effects of siltation and Grandmaison et al. (2003, pages 17-18) summarizes the information as follows: "Bhowmik and Adams (1989) provide an example of how sediment deposition has altered aquatic habitat in the Upper Mississippi River system where the construction of locks and dams has resulted in a successional shift from open water to habitats dominated by submergent and emergent vegetation. This successional process is not likely to favor species such as the crystal darter which rely on extensive clean sand and gravel raceways for population persistence (Page 1983). For example, the crystal darter was broadly distributed in tributaries of the Ohio River until high silt loading and the subsequent smothering of sandy substrates occurred (Trautman 1981). In the Upper Mississippi River, the relative rarity of crystal darters has been hypothesized as a response to silt deposition over sand and gravel substrates (Hatch 1998)".

Water Quality/Sewage Treatment

One common source of chemical water quality impairments is untreated or poorly treated wastewater (sewage). Although municipal wastewater treatment has improved dramatically since passage of the 1972 amendments to the Federal Water Pollution Control Act (Clean Water Act), some wastewater treatment plants, especially smaller plants, continue to experience maintenance and operation problems that lead to discharge of poorly treated sewage into streams and rivers (OEPA 2004 in USFWS 2008, page 23). Untreated domestic sewage (straight piping) and poorly operating septic systems are still problems within the Elk River watershed (WVDEP 2008, page 3; WVDEP 1997, page 54). Untreated or poorly treated sewage contributes a variety of chemical contaminants to a stream including ammonia, pathogenic bacteria, nutrients (e.g., phosphorous and nitrogen), and Biochemical Oxygen Demand (BOD), a measure of the speed at which bacteria consume oxygen in the water during breakdown of organic material (USFWS 2008, page 23). Two constituents from this list that may have the greatest impact on darters are nutrients and BOD. Excessive nutrients can lead to overabundant algal growth. Although algae produce oxygen while photosynthesizing during daylight, at night they respire (consume oxygen), and when they die, decomposition by aerobic bacteria consumes additional oxygen. Excessive BOD and nutrients in streams can lead to low dissolved oxygen (DO) levels in interstitial areas of the substrate. Low interstitial DO has the potential to be particularly detrimental to diamond darters, which live on and under the bottom substrates of streams and lay eggs in interstitial areas, both areas where a high level of decomposition and consequently, oxygen depletion, take place (USFWS 2008, page 23). Adequate oxygen is an important aspect of egg development and reduced oxygen levels can lead to increased egg mortality, reduced hatching success, and delaying hatching (Keckeis et al. 1996, page 436). Elevated nutrients in substrates can also make these habitats unsuitable for fish spawning, breeding, or foraging and reduce aquatic insect diversity which may impact availability of prey and ultimately fish growth (USFWS 2007, page 5). Darters are noted to be "highly sensitive" to nutrient increases associated with sewage discharges and studies have demonstrated that the abundance and distribution of darter species decreases downstream of these effluents (Wynes and Wissing 1981, page 259; Katz and Gaufin 1953, page 156). Elevated levels of fecal coliform signal the presence of improperly treated wastes (WVDEP 2008, page 7) that can cause these types of problems.

The reach of the Elk River from the mouth to river mile 27.7, which includes the area supporting the diamond darter, is currently on the State's 303(d) list of impaired waters due to violations of fecal coliform levels (WVDEP 2008b, page 1; WVDEP 2008a, page 18). There are noticeable increases in fecal coliform near population centers adjacent to the Elk River including Charleston, Elkview, Frametown, Gassaway, Sutton, and Clay (WVDEP 2008b, page 8). Elk River tributaries near Clendenin also show evidence of organic enrichment and elevated levels of fecal coliform (WVDEP 1997, page 48). The WVDEP notes that failing or non-existent septic systems are prevalent throughout the lower Elk River Watershed (WVDEP 2008, page 1). In order to address water quality problems, the WVDEP conducted a more detailed analysis of two major tributary watersheds to the lower Elk River. They found that all residences in these watersheds were "unsewered" (WVDEP 2008b, page 7). The Kanawha County Health Department Sanitarians estimate that the probable failure rate for these types of systems is between 25-30% and recent monitoring suggests it may be as high as 70% (WVDEP 2008b, page 7). In another study, it was noted that straight pipe/grey water discharges are often found in residences within the Elk River Watershed because the extra grey water would overburden septic systems. These untreated wastes are discharged directly into streams. Many household cleaning and disinfectant products could be components of this grey water and can harm stream biota (WVDEP 1997, page 54).

Impoundment

One of the reasons the diamond darter may have been able to persist in the Elk River is because the river remains largely unimpounded. Although there is one dam on the Elk River near Sutton, approximately 100 miles (161 km) of the river downstream of the dam retains natural free flowing riffle and pool characteristics, including the portion that supports the diamond darter (Strager 2008, page 5; USFWS, unpublished). All the other rivers with documented historical diamond darter occurrences are now either partially or completely impounded. There are four dams on the Green River, eight dams on the Cumberland, 11 locks and dams on the Muskingum, as well as a series of 20 locks and dams have impounded the entire Ohio River for navigation. Construction of most of these structures was completed between 1880 and 1950, however the most recent dam constructed on the Cumberland River was completed in 1973 (Clay 1975, page 3; American Canals 2009, page 1; Tennessee Historical Society 2002, page 4; Ohio Department of Natural Resources 2009, page 1; Trautman 1981, page 25). These impoundments have permanently altered habitat suitability in the affected reaches and/or fragmented stream habitats, blocking immigration and emigration between the river systems and preventing recolonization (Grandmaison et al. 2003, page 18). Trautman (1981, page 25) notes that the impoundment of the Muskingum and Ohio rivers for navigation purposes almost entirely eliminated riffle habitat in these rivers, increased the amount of silt settling on the bottom which covered former sand and gravel substrates, and affected the ability of the diamond darter to survive in these systems. In addition, almost the entire length of the Kanawha River including the 33 miles (53 km) above the confluence with the Elk River and an additional 58 (93 km) miles downstream to Kanawha's confluence with the Ohio River, has been impounded for navigation (U.S. Army Corps of Engineers 1994, pages 1, 13, 19). The series of dams and impoundments on this system likely impedes movement between the only remaining population of the diamond darter and the larger Ohio River Watershed including the other known historical river systems. Range fragmentation and isolation is noted to be a significant threat to the persistence of the diamond darter (Warren et al. 2000 in Grandmaison et al. 2003, page 18).

Direct Habitat Disturbance

There is the potential for direct disturbance, alteration, and fill of diamond darter habitat in the Elk River. Of particular note is a recent proposal to construct a bridge across the Elk River at Coonskin Park in Kanawha County, West Virginia. Coonskin Park is adjacent to one of the two reaches of the Elk River where diamond darters have routinely been captured. (Cincotta 2009c, page 1; Welsh 2009d, page 1) The West Virginia National Guard (WVNG) owns property adjacent to the park and visitors must pass through WVNG properties to access the park. The WVNG has identified this as a potential security concern and is proposing to construct a new access road to the park. Their currently preferred alternative includes a bridge across the Elk River (WVNG 2009, pages 1-2). Construction of this bridge would likely require direct disturbance and fill in the Elk River in one of the few reaches of the river known to support the species. Placement of bridge piers in the river would result in a permanent loss of habitat and could alter flows and substrate conditions, making the area less suitable to support the species (Welsh 2009d, page 1). The project is currently in the planning stage, but is expected to proceed within the next few years (WVNG 2009, page 3). The Service is currently working with the project proponent and other stakeholders to develop alternatives that avoid impacts to the river.

B. <u>Overutilization for commercial, recreational, scientific, or educational purposes</u>.

Due to the small size and limited distribution of the only remaining population, the diamond darter is potentially vulnerable to over-utilization. Particular care must be used to ensure that collection for scientific purposes does not become a long-term or substantial threat. It is possible that previous scientific studies may have impacted the population. Of the 19 individuals captured to-date, 14 either died as a result of the capture or were sacrificed for use in scientific or educational studies. The remaining five were removed from the system and are now being held for captive propagation work. It should be noted that there were valid scientific purposes for most of these collections. In order to verify the identification and permanently document the first record of the species in the State, the specimen captured in 1980 was preserved and vouchered consistent with general scientific protocols of the time. Subsequent surveys in the

1990s were conducted for the specific purpose of collecting additional specimens to be used in the genetic and morphological analyses required to determine the taxonomic and conservation status of the species. The extent and scope of these studies were determined and reviewed by a variety of entities including WVDNR, the USFWS, USGS, University scientists, and professional ichthyologists (Wood and Raley 2000, pages 20-26; Welsh and Wood 2008, pages 62-68; Lemarie 2004, pages 1-57; Tolin 1995, page 1). In addition, when these collections were initiated, insufficient data were available to establish the overall imperiled and unique status of the species. Because these studies are now complete, there should be limited need to sacrifice additional individuals for scientific analysis.

It is possible that future surveys conducted within the range of the species could inadvertently result in mortality of additional individuals. For example, during some types of inventory work fish captured are preserved in the field and brought back to the lab for identification. Young-of-the-year diamond darters are not easily distinguished from other species, and their presence within these samples may not be realized until after the samples are processed. This was the case during studies recently conducted by a local university. Future surveys should be designed with protocols in place to minimize the risk that diamond darters will be inadvertently taken during non-target studies. The WVDNR currently issues collecting permits for many surveys conducted within the State and could incorporate appropriate conditions into any permits issued for studies that will occur within the potential range of the species.

Although the species has no commercial value, it is possible that live specimens may be collected for the aquarium trade (Walsh et al. 2003 in Grandmaison et al. 2003 page 19), and that once its rarity becomes more widely known, it may become attractive to collectors. However, there is no information available to suggest that this is currently a threat.

C. Disease or predation.

There is no information available to suggest that disease or predation present an unusual threat to diamond darters. Although some natural predation by piscivorous fish and wildlife may occur, darters usually only comprise an almost incidental component in the diet of predators (Page 1983, page 172). This incidental predation is a normal aspect of the population dynamics and is not considered to currently pose a significant threat to the species. Commonly reported parasites and diseases of darters, in general, include metacercarial trematodes (black-spot disease) flukes, nematodes, leeches, spiny-headed worms, and copepods (Page 1983, page 173). None of the information provided regarding diamond darters captured to-date, or reports on the related crystal darter, note any incidences of these types of issues.

D. The inadequacy of existing regulatory mechanisms.

There are no existing federal or state regulatory mechanisms that specifically protect the diamond darter or its habitat where it currently occurs. Although the State of West Virginia prohibits taking species for scientific purposes without a permit, this requirement does not provide any protection to the species' habitat. There is no state equivalent to the federal Endangered Species Act.

The species is indirectly provided some protection from federal actions and activities through the Endangered Species Act, because the Elk River also supports three federally-listed mussel species. The reach of the Elk River currently known to support the diamond darter is also known to support the pink mucket. However, protective measures for listed freshwater mussels have generally involved surveys for mussel species presence and minimization of direct habitat disturbance in areas with confirmed presence. The diamond darter is more mobile and therefore is likely to be present within a less restricted area than most mussel species. Surveys for mussels will not detect diamond darters. As a result, these measures provide limited protection for the diamond darter.

Other existing authorities available to protect riverine ecosystems may not have been fully utilized, such as the Clean Water Act (CWA), administered by the Environmental Protection Agency, the U.S. Army Corps of Engineers (USACE), and State agencies. Since the CWA was enacted in 1972, strides have been made in meeting the Act's objective to "…restore and maintain the chemical, physical, and biological integrity of the Nation's waters." However, deficiencies in monitoring and enforcement, as well as the effects of recent court cases such as Rapanos and Carabell vs. USACE, have resulted in diminished effectiveness of CWA protections (USEPA 2001c, pages i-ii; U.S. Congress 2008, page 21).

E. Other natural or manmade factors affecting its continued existence.

Didymosphenia geminate

The presence of *Didymosphenia geminate*, a diatom commonly known as "didymo" or "rock snot" has the potential to adversely affect diamond darter populations in the Elk River. This species, native to portions of North America, has recently expanded its range, and has begun occurring in large nuisance blooms that can dominate stream surfaces by covering 100% of the substrate with mats up to 8 inches (20 cm) thick that can extend over 0.6 mile (1 km) and persist for several months (Spaulding and Elwell 2007, pages 3 & 6). Didymo can greatly alter the physical and biological conditions of streams in which it occurs, and cause changes to algal, invertebrate, and fish species diversity and population sizes; stream foodweb structure; and stream hydraulics (Spaulding and Elwell 2007, pages 3 & 12). Didymo is predicted to have particularly detrimental effects on fish, such as the diamond darter, that inhabit benthic habitats or consume benthic prey (Spaulding and Elwell 2007, page 15).

While didymo was previously thought to be restricted to cold water streams, it is now known to occur in a wider range of temperatures, and has been documented in waters that were up to 80°F (27°C) (Spaulding and Elwell 2007, pages 8, 10, & 16). It can also occur in a wide range of hydraulic conditions including slow moving, shallow areas, and areas with high depths and velocities (Spaulding and Elwell 2007, page 16-17). Didymo can be spread either through the water column or when items such as fishing equipment, boots, neoprene waders, and boats are moved between affected and unaffected sites (Spaulding and Elwell 2007, pages 19-20). Although it has not been documented to occur in the lower Elk River where the diamond darter occurs, in 2008 the WVDNR documented the presence of didymo in the upper Elk River, above Sutton Dam near Webster Springs (WVDNR 2008, page 1). Anglers have also reported seeing heavy algal mats in this reach of the river (WVDNR 2008, page 1). Therefore, there is potential that the species could spread downstream to within the current range of the diamond darter.

Genetic variation/Geographic Isolation
The existing diamond darter population is small in size and range, and is geographically isolated from other areas that previously supported the species. This patchy distribution pattern of populations in short stream reaches and small population size makes them much more susceptible to extirpation from single catastrophic events (such as toxic chemical spills or storm events that destroy habitat). It also reduces their ability to recover from smaller impacts to their habitat or populations. Furthermore, this level of isolation makes natural repopulation of unoccupied habitat impossible without human intervention.

Small, isolated populations may suffer from decreasing within-population diversity due to inbreeding among close relatives. This increases the likelihood of problems such as reduced fertility and fitness (Noss and Cooperrider 1994, page 61). Similarly, the random loss of adaptive genes through genetic drift may function to limit the ability of diamond darters to respond to changes in their environment (Noss and Cooperrider 1994, page 61). Small population sizes and inhibited gene flow between populations may increase the likelihood of local extirpation (Gilpin and Soulé 1986, pages 32-34).

CONSERVATION MEASURES PLANNED OR IMPLEMENTED

The U. S. Fish and Wildlife Service (Service), WVDNR, USGS, West Virginia University, and CFI are currently working together to implement conservation measures for the diamond darter. CFI in Tennessee is currently holding five individual diamond darters in captivity. They are conducting research on the reproductive biology and life history of the species and initiating attempts to - establish a captive “ark” population (a population maintained in captivity in order to avert extinction or preserve genetic diversity). In addition, over the next few years, the USGS West Virginia Cooperative Fish and Wildlife Research Unit at WVU will be conducting additional diamond darter surveys in the Elk River. If additional specimens are captured they will likely be transported to CFI, to supplement their propagation efforts. Some propagated individuals will likely be released back into the Elk River to supplement existing populations. These collections and releases should be conducted in accordance with protocols developed to ensure that these efforts do not threaten the viability of existing populations. Additional life history studies will also be conducted in conjunction with these two efforts. These projects are being funded by the Service and the WVDNR and appropriate scientific collecting permits are in place.

SUMMARY OF THREATS

The primary threats to the diamond darter are related to the present or threatened destruction, modification, or curtailment of its habitat or range. The species is currently known to exist only in the lower Elk River, West Virginia. This watershed is threatened with ongoing water quality degradation and habitat loss from activities such as coal mining, oil and gas development, siltation from these and other sources, inadequate sewage and wastewater treatment, and direct habitat loss and alteration through activities such as bridge construction. The impoundment of rivers in the Ohio River Basin, such as the Kanawha, Ohio, and Cumberland, has eliminated much of the species' habitat and isolated the existing population from other watersheds that the species historically occupied. Invasive species such as "didymo" have the potential to impact the Elk River and diamond darter habitat. The small size and restricted range of the remaining diamond darter population make it particularly susceptible to the effects of genetic inbreeding, as well as potential extirpation from spills and other catastrophic events. The species is vulnerable to over utilization for scientific purposes, however the significance of this threat has been reduced and can be further minimized through the administration of existing scientific collecting permit procedures. Existing federal and state regulatory mechanisms do not currently provide protections for the species or its habitat.

RECOMMENDED CONSERVATION MEASURES

The following list of recommended conservation measures was generated based on discussions with species experts, review of pertinent State Wildlife Conservation Action Plans, and the evaluation of threats as presented in this assessment. These proposed actions are not presented in any order of priority. However, management priority should be given to high quality habitat areas currently supporting diamond darter populations rather than on restoring other heavily impacted areas that currently do not support the species since the costs of restoring degraded habitats are high.

Surveys, Research, and Monitoring

- Establish standardized protocols and conduct regular monitoring of the diamond darter population and its habitat quality within the Elk River Watershed. Design these activities to minimize disturbances to diamond darter populations and habitats.

- Conduct additional life history studies focusing on reproduction, foraging, and habitat requirements.

- Conduct research to further quantify the effects of sedimentation and water quality changes to the survival and reproduction of the species.

- Develop an estimate of effective population size within the Elk River through non-lethal genetics analysis.

- Conduct additional surveys within remaining suitable habitat in the Cumberland River watershed and other areas within the Ohio River Basin. Surveys should use gear targeted towards capturing the diamond darter and should be designed to minimize potential impacts to the species' populations and habitats.

- Conduct habitat assessments within the species historical range to determine if other areas of potentially suitable habitat exist. Conduct baseline surveys within identified areas of potentially suitable habitat.

Habitat Protection and Restoration

- Implement habitat protection and restoration measures within the Elk River Watershed. This could include efforts to protect and restore riparian habitat; maintain or improve water quality; reduce sedimentation and erosion; increase stream bank stability; avoid activities that result in channel modifications or disturbance; improve wastewater treatment systems; develop and implement improved land management, forestry, and agricultural practices; reduce the impacts of industrial and residential development; reduce the impacts of coal mining and oil and gas development; and reduce nutrient and/or pollution loads from non-point and point source discharges. Measures to provide long-term protection for key habitat areas within the watershed through purchase from willing landowners or the establishment of conservation easements should also be pursued.

- Implement habitat protection and restoration measures within watersheds in the species historical range but where the species is thought to be extirpated so that these areas become suitable to support the species.

Captive Propagation and Reintroduction

- Develop captive propagation and holding techniques.

- Establish protocols for ensuring additional collections do not affect viability of existing populations.

- Develop and maintain a captive "ark population" in order to avert potential extinction in the event of major spill event or other threat on the Elk River.

- Develop reintroduction protocols and criteria, and evaluate sites for potential reintroduction.

- Supplement existing populations with captive-reared darters.

- Establish additional populations by reintroducing the species into suitable habitat within the historical range.

Education and Outreach

- Conduct outreach and education efforts targeting landowners and other stakeholders within the Elk River Watershed that highlight the importance of the Elk River, the diamond darter, and the need to maintain high water and habitat quality.

- Initiate and/or support efforts to develop watershed-based partnerships that incorporate a diverse group of stakeholders who will help assess and implement watershed protection and restoration measures within the Elk River or other watersheds that contain potentially suitable diamond darter habitat.

LISTING PRIORITY

THREAT			
Magnitude	Immediacy	Taxonomy	Priority
High	**Imminent**	Monotypic genus	1
		Species	**2***
		Subspecies/population	3
	Non-imminent	Monotypic genus	4
		Species	5
		Subspecies/population	6
Moderate to Low	Imminent	Monotypic genus	7
		Species	8
		Subspecies/population	9
	Non-imminent	Monotypic genus	10
		Species	11
		Subspecies/population	12

Rationale for listing priority number:

Magnitude: The threats to the diamond darter are at a very high magnitude, in that the entire range of the species is potentially affected. The species is currently known to exist in only one watershed, the Elk River in West Virginia. Threats to this watershed include coal mining, oil and gas development, siltation, and inadequate sewage treatment. These threats are pervasive throughout the watershed. Because of its restricted range, the species is extremely vulnerable to extinction as the result of a spill or other catastrophic event.

Immediacy: The threats to the species are imminent and ongoing. Activities such as coal mining, oil and gas development, and inadequate sewage treatment facilities already exist within the watershed and are expected to continue. Planning to construct a bridge in one of the two reaches of the Elk River that are known to support the species is underway and construction may be expected in the next few years.

Yes Have you promptly reviewed all of the information received regarding the species for the purpose of determining whether emergency listing is needed?

Is Emergency Listing Warranted? Emergency listing is not warranted at this time. Although the magnitude and immediacy of threats to the diamond darter are high, previous monitoring over the past 20 years has shown the species continues to exist, although likely in low numbers. If conservation actions can be initiated in a timely manner, the continued existence of the species or loss of significant remaining recovery potential should not be compromised. However, we are concerned about plans to construct a bridge across the Elk River to access the WVNG property near Coonskin Park that would potentially cause direct loss and alteration of habitat. As the planning for this project proceeds, we will consider how it affects the magnitude and immediacy of threats to the diamond darter.

DESCRIPTION OF MONITORING

There are currently no programs established for monitoring the diamond darter. Although some additional surveys will occur within the Elk River in the near future, as described under the "Conservation Measures Planned or Implemented," there is no long-term funding available to continue these efforts. Occasional surveys may be conducted in and around the historic range of the species as part of monitoring or research being conducted by States or other entities.

In order to track the extent of ongoing threats to the diamond darter, the Service and the WVDNR will continue to monitor project proposals that may affect the Elk River and work with project proponents to avoid and minimize impacts. Coordination with species experts and other biologists and managers will continue to be conducted on a routine basis in order to determine if additional or emergency recovery or listing measures will need to be enacted.

COORDINATION WITH STATES

The following State agencies provided comments or information that contributed to this species assessment: West Virginia Division of Natural Resources, West Virginia Department of Environmental Protection, West Virginia University/West Virginia Cooperative Fish and Wildlife Research Unit, Kentucky State Nature Preserves Commission, Ohio Environmental Protection Agency, Ohio State University Museum of Biological Diversity, Tennessee Valley Authority, and the Tennessee Wildlife Resources Agency. The diamond darter is included as a species of conservation concern in both the West Virginia and Tennessee State Wildlife Action Plans (WVDNR 2005, page 503; TWRA 2005, Apdx. B-3).

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APPROVAL/CONCURRENCE: Lead Regions must obtain written concurrence from all other Regions within the range of the species before recommending changes, including elevations or removals from candidate status and listing priority changes; the Regional Director must approve all such recommendations. The Director must concur on all resubmitted 12-month petition findings, additions or removal of species from candidate status, and listing priority changes.

Approve:______________________________ Date: 5/26/10
Acting Regional Director, U.S. Fish and Wildlife Service

Concur: ______________________________
ACTING
Director, Fish and Wildlife Service Date: October 22, 2010

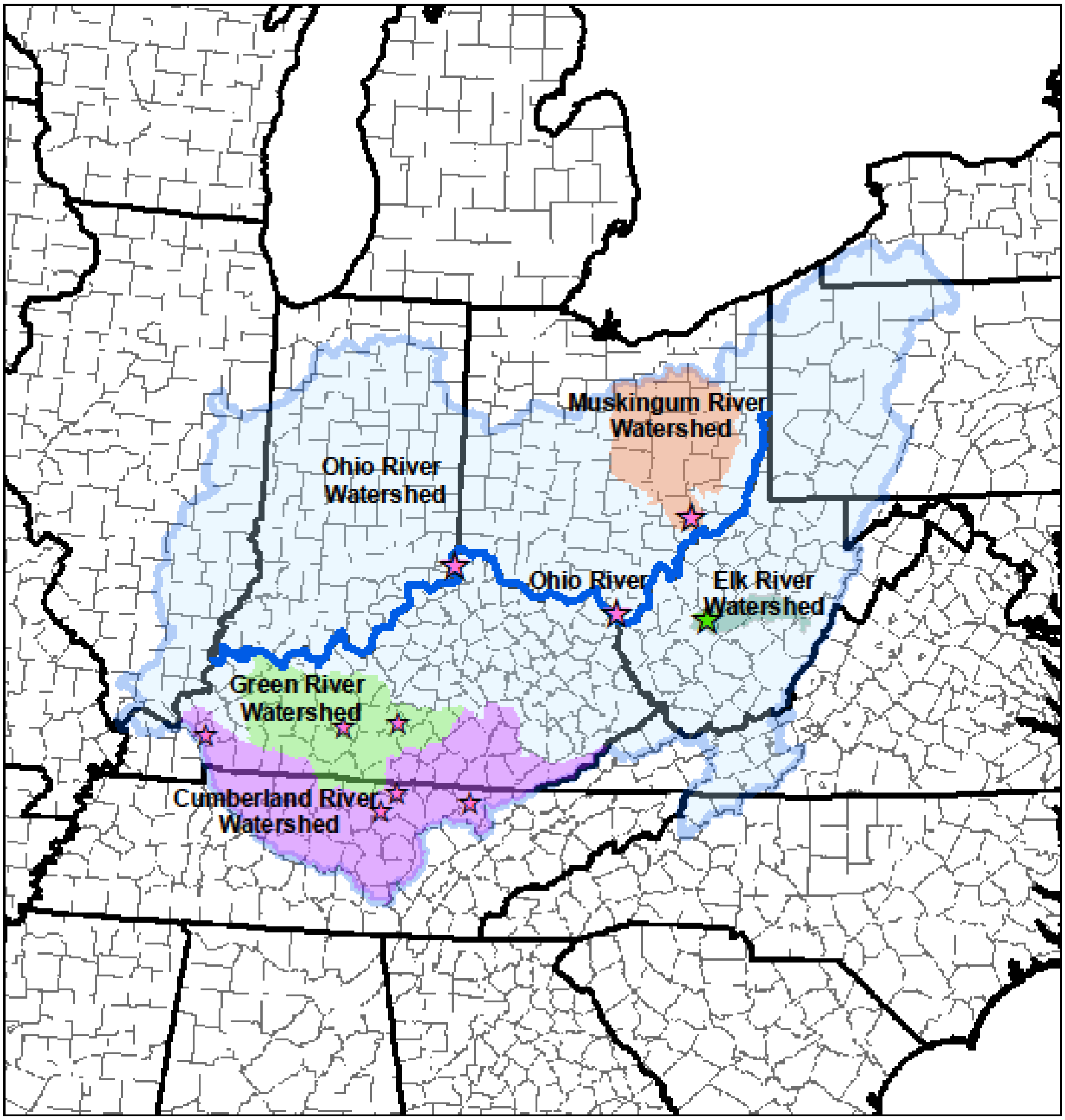
Do not concur: ______________________________
Director, Fish and Wildlife Service Date

Director's Remarks:

Date of annual review:
Conducted by:

Figure 1:

Historic and Current Range of the Diamond Darter



Current Capture Locations

Historic Capture Locations

Figure 2: Distribution of Mining Activities in the Elk River Watershed

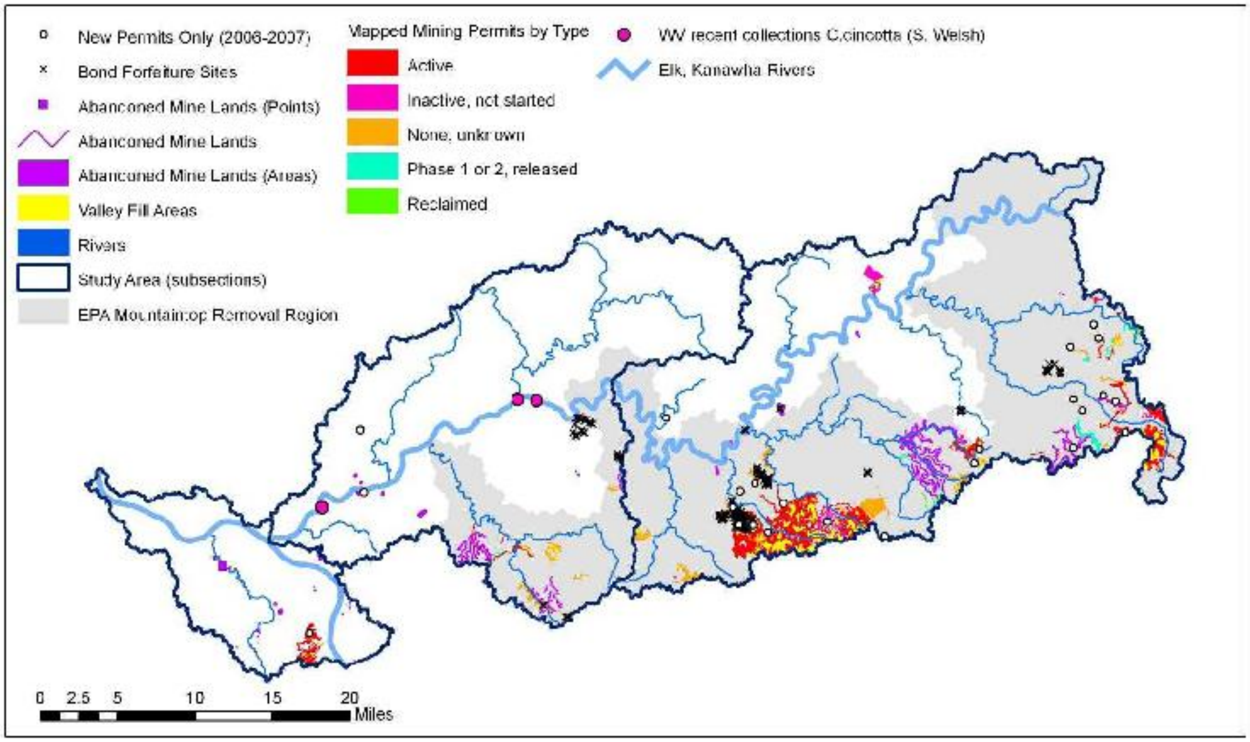


Figure 3: Distribution of Oil and Gas Activities in the Elk River Watershed